Article

Journal of Biomedical Nanotechnology
Vol. 9, 659–663, 2013
www.aspbs.com/jbn

Label-Free Nanobiosensor to Detect Infectious Bacterica Based on SERS

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Bacteria detection has been important in food, water and air industry to detect contamination or infection. However, conversational bacteria detection method such as culture collection is time-consuming and complicated. In this study, Surface Enhanced Raman Scattering (SERS), one of the most convenient and reliable biosensing techniques, was applied to detect bacteria such as *e. coli*. First, gold nanoparticles were deposited on Indium Tin Oxide (ITO) substrate by using electrochemical method and confirmed by Scanning Electron Microscope and Ultraviolet-Visible spectroscopy. After antibody against *e. coli* was immobilized on the gold nanoparticles fabricated on ITO substrate, *e. coli* samples with different concentrations were applied to the system by antibody-antigen interaction. Then, *e. coli* was detected by using SERS spectroscopy, demonstrating that the SERS peak intensity at certain raman shift were good relationship with the concentration. In conclusion, this technique could be applied to the biosensor or biochip such as raw milk chip.

KEYWORDS: *Bacteria, Surface Enhanced Raman, E. Coli, Nanoparticle, Biosensor.*

INTRODUCTION

All humans are infected with bacteria living on their external surfaces (including the skin, gut and lungs). We are constantly also exposed to bacteria (including air, water, soil and food). First, bacteria infected to intestines of cow or other ruminants. Then, these bacteria infected to meat, diary products or water and vegetables for a second. Finally, human are infected with bacteria from taking infected food.[1] Thus, it is important to diagnose bacteria fast at the place that is infected with bacteria at first time such as farm.

Bacteria are a large domain of prokaryotic microorganisms.[2] The bacterial cell is surrounded by a lipid membrane, or cell membrane, which encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell.[2] Bacteria are composed of gram positive bacteria and gram negative bacteria. In gram positive bacteria, no outer membrane exists whereas outer membrane exists in gram negative bacteria.[3]

There is a definite method to detect bacteria from culture. It is obvious method to detect bacteria, but it consumes too much time about 3–4 days and is complicated to detect.[4] The mechanism of the bacteria culture is multiplying microbial organisms using growth medium.[4] Microbiological cultures can be grown in petri dishes of differing sizes that have a thin layer of agar-based growth medium. However, the limit of bacteria culture is long time to detect and complicated. Moreover, ELISA is very commercial way and easy to detect material, but using label is the weakness in ELISA.[5] Also immuno-PCR is very exact way to detect but it consumes too much time and is complicated to detect biomaterial.[6] Also, there are some problems in bio-barcode method such as long assay time, complicated to detect and low reproducibility.[7] Thus, simple and fast method to detect bacteria is required now.

Biosensor based on SERS can be promising biosensor in future. Because SERS is enhancement of raman, biosensor based on SERS can detect biomaterials with

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Received: 14 October 2011
Accepted: 4 March 2012

ultrasensitive level.[8] Also, labeling is not needed when detecting biomaterials based on SERS. So, biomaterial can detect with label-free using SERS. Raman band shows the molecular structure efficiently and exactly. So biomaterials can be detected with efficient and ultrasensitive using SERS because molecular finger printing is capable.[8]

Hence, in this study, very low concentration of gram negative bacteria *e. coli* and gram positive bacteria listeria monocytogenes were detected based on SERS technique range from 10^1 CFU/ml to 10^5 CFU/ml.

EXPERIMENTAL DETAILS

Materials

2×2 cm Indium Tin Oxide (ITO) substrate was purchased from G-Mac (Korea). Hydrogen tetrachloaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Sigma Aldrich (USA) and 3-[(3-Cholamidopropyl) dimethyl ammionio-]-1-propane sulfonate (CHAPS) was purchased from Roche (USA) to fabricate gold nanoparticles on ITO substrate. 11-Mercapto Undecanoic Acid (11-MUA) purchased from Sigma Aldrich (USA) was used as a starting material of Self-Assembled Monolayers (SAMs). 1-Ethyl-3-[3-dimethyl aminopropyl] carbobiimide (EDC) and N-hydrxysuccinimide (NHS) purchased from Thermo (USA) was used as linker between antibody and gold nanoparticles fabricated on ITO substrate. *E. coli* and Listeria Monocytogenes were purchased from Culture Collection of ANTIMICROBIAL Resistant Microbes (CCARM). Ammonium Hydroxide and hydrogen peroxide purchased from Dae-Jung were used as basic piranha solution. Distilled and deionized Milipore [(Milli-Q) water (DDW>8 MΩ)] and nitrogen gas were used for cleaning and drying. And all other chemicals were purchased from the Aldrich Chemical Co. (USA) and used without further purification.

Fabrication of Gold Nanoparticles on ITO Substrate

Gold nanoparticles were deposited on ITO substrate by electrochemical method. Prior to fabrication of gold nanoparticles on substrate, ITO substrate was washing with 1% of Triton-X solution, DI water and Ethanol sequentially using sonication for about 45 minutes at each solution. And this substrate were treated with basic piranha solution (DI water:NH_4OH:H_2O_2 = 5:1:1 v/v) for 30 min at 80 °C to make hydroxyl group on the surface. Then, ITO substrate was immersed in the solution which consisted of 2 mM $HAuCl_4$ and the optimized concentration of CHAPS.[9–10] To fabricate gold nanoparticles on ITO substrate, amperometry was used based on three-electrode system. Thus, ITO substrate was connected to the working electrode in this gold and CHAPS solution. And gold nanoparticles were deposited on ITO substrate at −1.3 V (Ag/AgCl) at 25 °C.[10]

Immobilization of Target Material on Gold Nanoparticle Fabricated on ITO

Gold nanoparticle fabricated on ITO substrate were immersed in 10 mM 11-MUA in ethanol at 4 °C overnight and washed with ethanol and DI water to eliminate non-bonded thiols.[20] Then, Gold nanoparticle fabricated ITO substrate were applied to the 0.1 M of EDC/NHS solution to make aldehyde group on the surface of the substrate for 1 hour at room temperature. After washing with DI water and PBS buffer, the substrate was incubated with 10 μg/ml of antibody against target material at 4 °C for 4 hours. Then, the substrate was washed with DI water and PBS buffer. Target material was applied to the substrate at room temperature to react with the antibody immobilized on the gold nanoparticles fabricated on ITO substrate. After 2 hours, the substrate was washed with PBS and DI water. Finally, target material was immobilized on the substrate. Thus each different concentration of the bacteria solutions from 10^1 CFU/ml to 10^5 CFU/ml was immobilized on the substrate for the measurement.

Detection of Bacteria Based on SERS

Bacteria solutions immobilized on the gold nanoparticles fabricated on ITO substrate were detected by SERS spectra using NTEGRA spectra (NT-MDT, Russia) that was equipped with a liquid nitrogen-cooled CCD detector.[11] The maximum scan range, XYZ, was $100 \times 100 \times 6$ μm, and the resolutions of the spectrometer in the XY plane and the Z axis were 200 nm and 500 nm, respectively. The SERS spectra were obtained using infrared-laser-emitting light at a 785 nm wavelength with a nirraidation laser power of 3 mW on the sample plane. Five 5 sec scans of 200-1, 800 cm^{-1} were recorded, and the mean intensity was used as the SERS signal.[20]

RESULTS AND DISCUSSION

Morphology of Gold Nanoparticle Fabricated ITO Substrate

By using three-electrode system which is composed with counter, reference and working electrode, gold nanoparticles were fabricated on ITO electrode.

The mechanism of this method is that the positively charged gold ions in aqueous solution will attach to the negative charged surface of the ITO substrate. When positively charged gold ions approaches to the substrate, the surfactant CHAPS will modify the interfacial properties of both the gold nanoparticles and the electrodes and to control the morphology of the aggregates. SEM image of the electrodeposited gold nanoparticles on an ITO substrate (Fig. 2) clearly demonstrates that, this electrodeposition results in surfaces with a well distribution of gold nanoparticles arranged in regular and good balance with small size and big size.

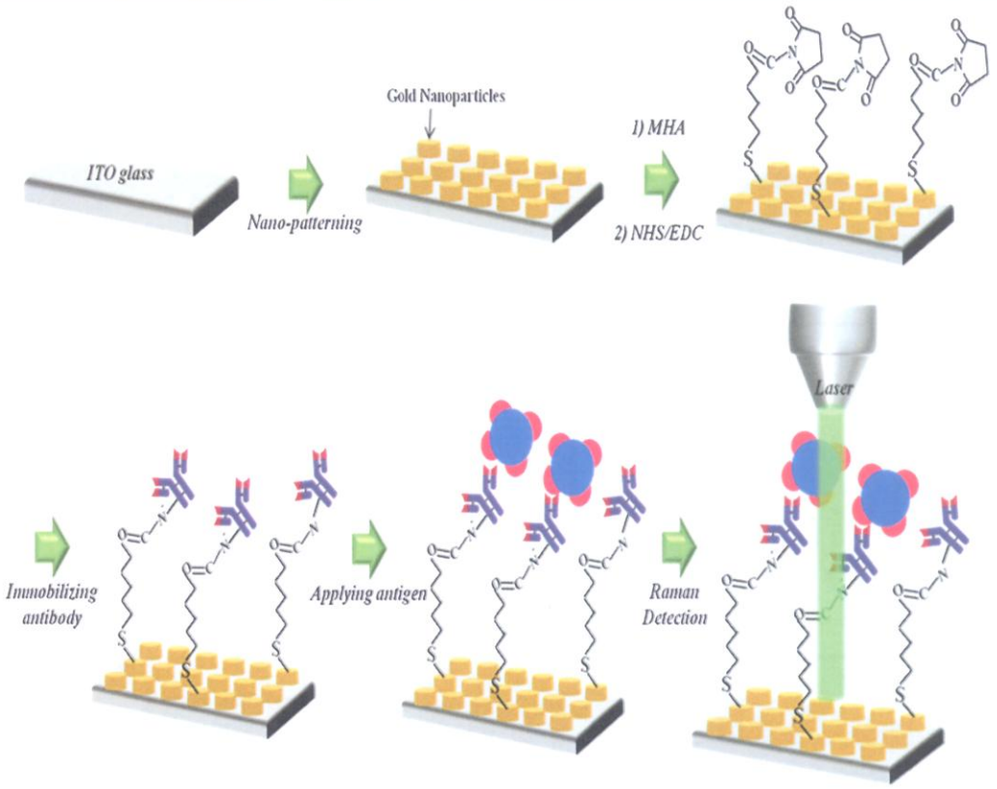

Figure 1. Schematic diagram of bacteria detection system based on SERS.

Comparison Between ITO Substrate and Gold Nanoparticles on ITO

Gold nanoparticles were fabricated on ITO substrate by electrochemical method, and then the gold nanoparticles fabricated on ITO substrate were measured by UV-vis spectrometer and raman spectrometer. The absorption spectra of gold nanoparticles fabricated ITO substrate demonstrates a weak surface plasmon absorption band within the visible region at around 570 nm contributed by transverse electronic oscillation, while bare ITO substate has no peak appearance (Fig. 3). Because the enhancement of surface electric field depends on the surface plasmon excitation, gold nanoparticles may strongly absorb the energy and scatters the electromagnetic field. Hence, it could be expected that gold nanoparticle fabricated ITO substrate may lead a high enhancement of raman signals compared to that of bare ITO substrate.

Detection of Bacteria Based on SERS

To develop highly sensitive and fast bacteria biosensor, two different bacteria one from gram negative (*E. coli*) and the other from gram positive (Listeria monocytogenes) bacteria were detected based on SERS technique.

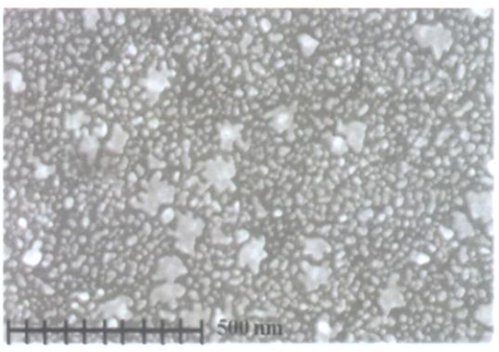

Figure 2. SEM image of gold nanoparticle fabricated ITO substrate.

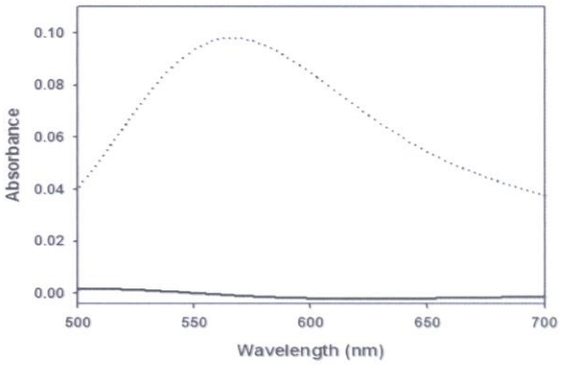

Figure 3. UV–vis spectra of (–) bare ITO substrate and (•••) gold nanoparticles fabricated ITO substrates

First, *E. coli*, one of major gram-negative bacteria has been measured. Some of *E. coli* are safe but some serotypes can cause serious food poisoning in human.[12] Figure 4(A) shows the SERS spectrum of the *E. coli* and the antibody against *E. coli*. The intensity and peak of SERS spectrum had a big difference between antibody against *E. coli* and *E. coli*. The SERS spectrum of antibody had almost linear, having no peak. The peaks at 616.33 cm^{-1} and 1321 cm^{-1} were assigned to C–C aliphatic chain vibrations and 1443.55 cm^{-1} and 1583.45 cm^{-1} were assigned to the lipids of cell wall of *E. coli*. Also, the peaks at from 600 cm^{-1} to 800 cm^{-1} were assigned to the nucleic acids structure of *E. coli*.

The SERS spectrum was recorded at different concentrations of *E. coli* range from 10^1 CFU/ml to 10^5 CFU/ml in Figure 3.10(B). The intensity was increased as the concentration of *E. coli* was increased, showing the

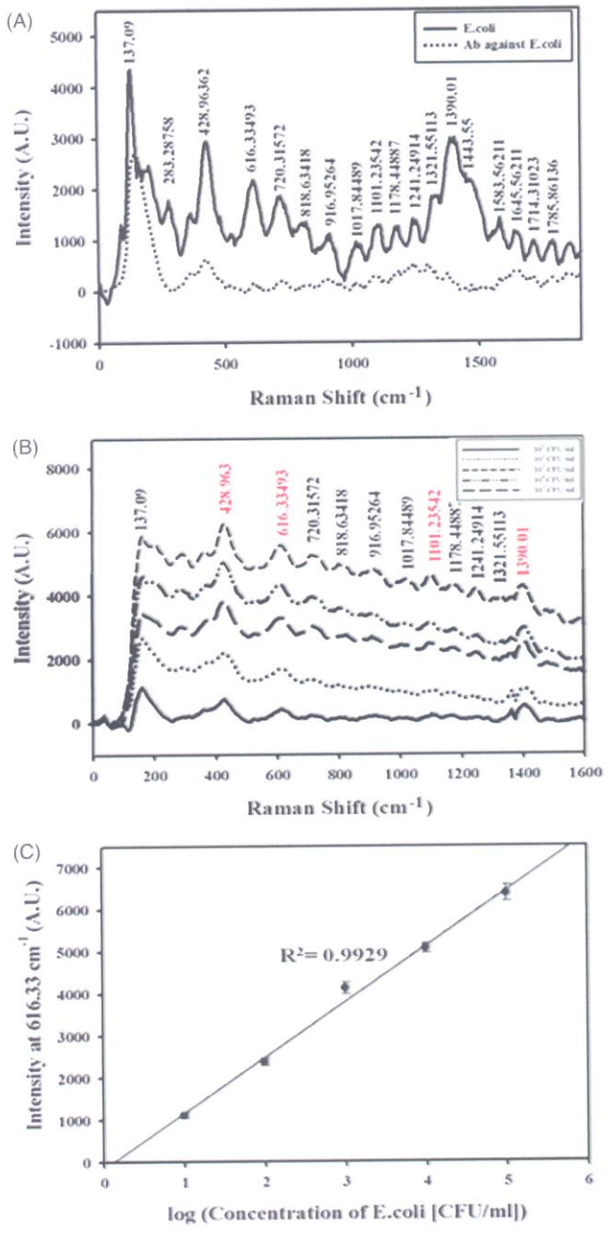

Figure 4. Representative SERS spetra of (A) Biosurface immobilized with (–) antibody and target (•••) *E. Coli*, (B) Different Concentrations of *E. coli* (–) 10^1 CFU/ml, (•••) 10^2 CFU/ml, (––) 10^3 CFU/ml, (– •• –) 10^4 CFU/ml, (— —) 10^5 CFU/ml, (C) Linear Plot of the SERS Peak Intensity at 616.33 cm^{-1} as a Function of the log concentration of *e. coli*.

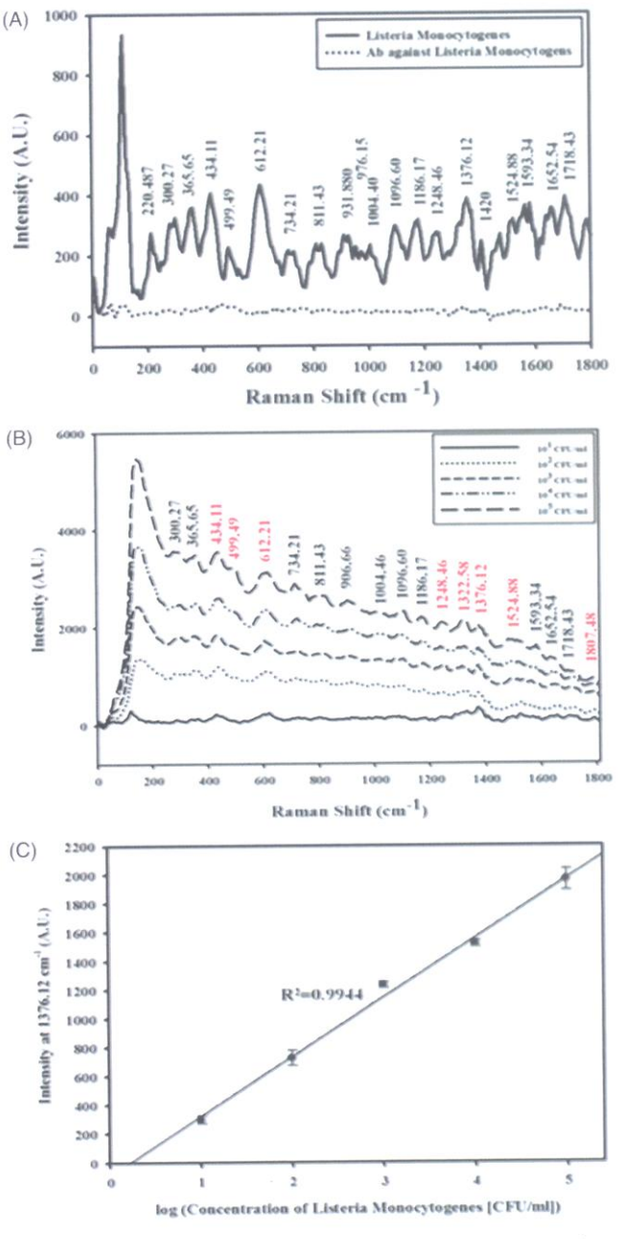

Figure 5. Representative SERS spetra of (A) Biosurface immobilized with (–) antibody and target (•••) Listeria Monocytogenes, (B) Different Concentrations of *E. coli* (–) 10^1 CFU/ml, (•••) 10^2 CFU/ml, (––) 10^3 CFU/ml, (– •• –) 10^4 CFU/ml, (— —) 10^5 CFU/ml, (C) Linear Plot of the SERS Peak Intensity at 616.33 cm^{-1} as a Function of the log concentration of *e. coli*.

obvious peaks of bacteria. Figure 4(C) shows the linear relationship between the concentration of *E. coli* and SERS intensity at 616.33 cm^{-1}. The SERS intensity was proportional to the logarithmic concentration of *E. coli* with $R^2 = 0.9929$.

Moreover, one of major gram-positive bacteria listeria monocytogenes was also measured, whose cell wall is composed of several layers of peptidoglycan and one of the hazardous bacteria resulting in fever, headache and convulsion.[13] Figure 5(A) shows the SERS spectrum of listeria monocytogenes. Compared to the raman band of antibody against listeria monocytogenes that has no specific raman peak, the raman band of listeria monocytogenes has obvious raman peaks at some specific raman shift. The peaks at 300.27 cm^{-1} and 365.65 cm^{-1} were assigned to C–C aliphatic chains. And the peaks which was assigned to alicyclic chains were confirmed (621.21 cm^{-1}, 734.21 cm^{-1}, 811.43 cm^{-1}, 931.88 cm^{-1}, 976.15 cm^{-1}, 1004.40 cm^{-1}, 1096.60 cm^{-1}, 1186.17 cm^{-1}, 1248.46 cm^{-1}, 1376.12 cm^{-1}) because cell wall of listeria monocytogenes were made of some alicyclic chains for making several pepidoglycan layers.

Figure 5(B) shows that the SERS intensity was increased with increasing the concentration of listeria monocytogenes. The same peak appeared despite the different concentration of listeria monocytogenes. From here, the intensity of the peaks at 1376.12 cm^{-1}, which is conrresponded to the ($C–NO_2$), was selected to study the relationship between the concentration of listeria monocytogenes and the SERS intensity. Figure 3.11(C) shows that the concentration of listeria monocytogens was linear relationship with the SERS intensity with $R^2 = 0.9944$.

CONCLUSION

Infectious bacteria such as *e. coli* (gram negative) and listeria monocytogenes (gram positive) were detected based on SERS. Gold nanoparticles were deposited on ITO substrate using electrochemical method to enhance the raman signal. Then, different concentrations of *e. coli* and listeria monocytogenes from 10^1 CFU/ml to 10^5 CFU/ml were detected based on SERS with linear relationship between the SERS intensity and the concentration of bacteria with $R^2 = 0.99$. Hence, our detection system could be usefully applied to develop highly sensitive and fast bacteria sensor in the various fields such as food and environments.

Acknowledgment: This work was supported by the Sogang University Research Grant of 2012 SRF.

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